

The University of Chicago

SPLENIC VOLUME AND THE
POLYCYTHEMIA OF ARTIFICIAL FEVER
IN INTACT, UNANESTHETIZED DOGS

A PART OF A DISSERTATION SUBMITTED
TO THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1938

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LAURENCE L. PALITZ

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SPLENIC VOLUME AND THE POLYCYTHEMIA OF ARTIFICIAL FEVER IN INTACT, UNANESTHETIZED DOGS

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Many authors have described the polycythemia occurring in dogs during experimental artificial fever (1, 2, 4). An increase in the formed elements and plasma concentration of blood has been reported in both experimental infectious fever and in fever induced with high frequency currents (3). The mechanism for this polycythemia is generally explained as a concentration of blood owing to surface loss of water (chiefly from panting). Most of the experiments have been conducted over long periods of time and under conditions of anesthesia which might have influenced the concentration of blood elements. The studies about to be described were made in an effort to ascertain the true blood picture in intact, unanesthetized dogs with artificial fever of short duration, and to determine as far as possible the physiological processes involved.

Banus and Ginsburg (4), in experiments with infectious fever, noted a much greater increase in the formed elements than could be explained by a concentration of plasma. They suggested that this increase might be due to a liberation of red cells from a storage reservoir, possibly the spleen, as well as to surface loss of water. Barcroft and Elliot (3), using the exteriorized spleen technique, found that when dogs were placed in a heated chamber (47—54.5°C.) the normal spleen contracted (15 per cent) and the denervated spleen dilated (3—11 per cent). Further, a steadily increasing amount of evidence points to the fact that contraction or dilatation of the spleen under natural stimuli or drug action can cause highly significant changes in the red cell count of peripheral blood. Von Haam (6) demonstrated that normal dogs, when subjected to intravenous epinephrine injections, showed an acute polycythemia; splenectomized dogs did not. Recently, Hausner, Essex and Mann (7) showed that the spleen contracts with ether anesthesia, causing an increase as high as 1,800,000 per cu. mm. in red cells in the peripheral circulation; with amyltal and the barbiturates it dilates, causing decreases as much as 2,500,000 per cu. mm.

The above investigators, therefore, have demonstrated (1) that polycythemia occurs as a result of artificial fever, and (2) that splenic volume varies enough to cause concentration of formed elements in the periph-

eral circulation. The following investigation is an attempt to relate the above facts so as to determine whether or not the splenic volume decrease is the principal cause of the changes noted.

PROCEDURE AND METHODS. Artificial fever was induced in dogs by means of a General Electric Inductotherm.¹ This instrument was found to be ideally suited to animal experimentation, because the temperature could be readily controlled and the apparatus easily applied.

Continuous spleen volume records were obtained on intact, unanesthetized dogs under normal and artificial fever conditions using the method previously described (12). Dogs with chronic, subcutaneous spleens, prepared as described, were given the inductotherm treatment. A water manometer attached to a sphygmomanometer cuff wrapped around the spleen of the animal simultaneously recorded changes in spleen volume on a kymograph drum. In animals thus prepared, induction of fever caused a significant contraction of the spleen.

A series of normal animals were then subjected to treatment and blood studies were made. After the dog, trained to lie quietly, had been placed on a table, 30 minutes were allowed for adjustment to the new environment (thus ruling out emotional and exercise effects on the spleen). A sample of normal blood (10 cc.) was then drawn from the saphenous vein. The rectal temperature was taken, and fever produced by means of the inductotherm. This procedure was repeated after half an hour and again after a full hour of fever treatment. The machine was turned off, and, after another 30 minutes, the last sample was procured. The temperature increases varied slightly in different experiments, but the range of increase was always from 2 to 4° Fahrenheit.

To determine whether the resulting polycythemia was absolute or relative, red cell counts, hematocrit readings, and serum protein determinations were made on the blood samples. A few cubic centimeters of blood were placed in a small heparin tube, and red cell counts as well as hematocrit readings (Wintrobe) were made from the sample. The remainder of the blood was placed in a centrifuge tube and serum obtained for protein determinations. These were made by the total nitrogen method of Van Slyke with the Koch-McMeekin modification (8, 9).

After a series of experiments on a given dog, the dog was splenectomized, and the same determinations were made with the same procedures in another series of experiments after recovery of the animal (table 1).

RESULTS. Figure 1 shows the composite results of four normal dogs given fever treatments as above described. With the onset of artificial fever, there were significant increases in red cell count, hematocrit (cell

¹ I am indebted to Mr. H. J. Holmquist of the G. E. X-Ray Corporation for the use of the inductotherm.

volume), and serum proteins. In a typical case, the red cell counts increased over the normal by 25 per cent, the hematocrit by 14.5 per cent, and the serum protein by 12.5 per cent. Similar changes occurred in all dogs studied, though the amount of increase varied from animal to animal.

From these results alone, it would appear that both an absolute *and* a relative increase in the formed elements of the blood had occurred. Concentration of blood by surface loss of water (panting of the animal) would

TABLE I

*Four experiments on dog 4, showing actual data obtained from the normal animal—
and from the same animal after splenectomy*

DATE	TIME	T°F.	HEMATOCRIT	RBC	SERUM PROTEIN
Normal					
1938			<i>cell vol.</i>	<i>millions</i>	<i>gr./100 cc.</i>
January 24.....	10:50*	101.4	34.0	5.110	5.78
	11:20	102.6	38.0	5.070	5.81
	11:50†	103.8	41.2	6.990	6.38
	12:20	101.8	37.0	4.990	5.77
January 31.....	9:50*	99.9	29.2	4.260	5.53
	10:20	102.4	35.6	4.925	6.14
	10:50†	103.7	40.0	5.410	6.80
	11:20	101.7	34.5	4.830	6.03
Splenectomized					
February 16.....	9:40	100.4	36.0	4.940	6.35
	10:10*	102.0	36.2	5.420	6.41
	10:40	103.0	36.0	5.000	6.25
	11:10†	101.0	36.3	4.805	5.69
February 23.....	10:30	100.6	33.9	4.995	5.66
	11:00*	101.6	33.8	5.090	5.56
	11:30	102.5	35.4	5.220	5.81
	12:30†	101.3	35.2	4.945	5.80

* Inductotherm turned on after first sample.

† Inductotherm turned off after third sample.

explain that part of the increase in red cells which might be correlated with increase in serum concentration. The increase of R. B. C. above the level of serum concentration might be due to an influx of red cells from a storage reservoir in the animal. But in the splenectomized animals there was no significant change in the formed elements, and surprisingly, no change in the concentration of plasma. Thus it would seem that in these short duration experiments the surface loss is negligible and the all-

important factor is the liberation into the general circulation of red cells from the spleen.

There is still to be explained the increase of serum proteins in the normal animals. It is impossible to assume that this increase is due to a concentration factor, because the splenectomized animals behaved in the same way (panting) as did the normal animals. Two possibilities present themselves: 1, that the spleen liberates serum with concentrated protein together with the formed elements, and 2, that the increased red cell content in the circulation (due to contraction of the spleen) in some way causes a concentration of serum.

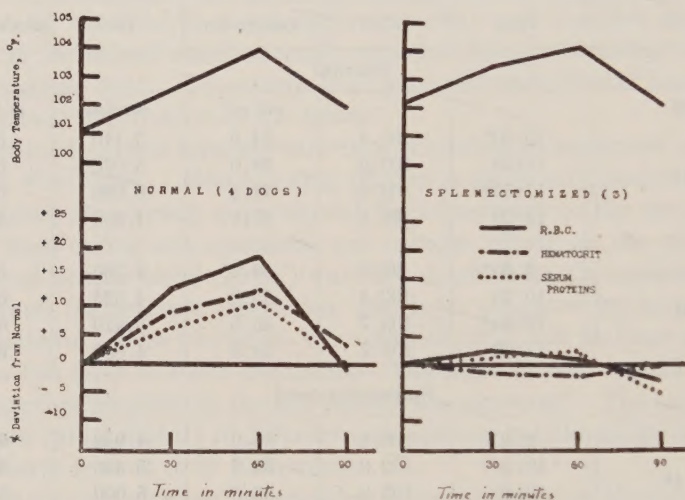


Fig. 1. Composite results of the effect of artificial fever treatment on the red cell count, hematocrit and serum protein in four dogs (19 experiments) and in three of the same dogs after splenectomy (11 experiments).

To explore these possibilities, acute experiments were done under anesthesia. After removing the spleen of a dog, concentrated blood from another dog was injected at intervals, in an attempt to simulate experimentally a splenic contraction. The donor blood was first centrifuged, most of the serum discarded, and the concentrated residue injected. Samples of blood were drawn ten minutes after each injection and the previously described determinations (red cell count, hematocrits and serum proteins) were carried out. Corrections were made for the small amount of protein injected.

Table 2 gives the results of these experiments. In every case, an absolute increase in formed elements, produced experimentally, caused a

concentration of serum proteins. This concentration appears to be approximately proportional to the increases in hematocrit reading, though a quantitative relationship has not yet been determined.

TABLE 2

Results of six acute experiments in which blood, concentrated in red cells, was transfused at intervals in splenectomized dogs, indicating the compensatory concentration of serum with absolute increases in cell volume

DATE	WEIGHT	BLOOD INJECTED	DONOR BLOOD	HEMATO- CRIT	INC.	RBC	INC.	SERUM PROT.	PROT INC.
	kgm.	cc.	per cent cells	per cent cells	per cent	million	per cent	gr./100	per cent
1/23/38	7.8	Normal		50.0		6.54		6.23	
		50.0	87.0	55.3	10.6	7.60	16.0	6.60	6.0
1/28/38	10	Normal		62.0		7.52		6.65	
		50.0	89.0	66.5	7.2	8.05	7.0	6.83	2.7
		50.0	89.0	70.5	13.7	9.53	26.7	7.28	9.5
2/ 1/38	20	Normal		55.3		7.44		6.60	
		25.0	81.0	61.2	10.6	7.99	7.4	6.86	3.9
2/ 9/38	14	Normal		50.0		7.90		6.28	
		25.0	80.0	56.3	11.2	8.49	7.2	6.45	2.8
		25.0	80.0	58.2	16.2	9.99	26.2	6.53	4.0
		25.0	80.0	61.0	22.0	10.95	38.0	6.60	5.0
		25.0	80.0	63.0	26.0	11.45	42.0	6.60	5.0
2/16/38	7.8	Normal		50.5		7.36		6.13	
		50.0	95.0	54.4	7.7	8.09	9.9	6.75	9.8
		50.0	95.0	55.6	10.1	8.76	18.8	6.96	13.7
2/24/38	10	Normal		46.4				5.66	
		100.0	97.0	55.6	19.9			6.26	10.4
		50.0	97.0	61.6	32.9			6.49	14.4
		50.0	97.0	66.3	42.9			6.79	19.9

DISCUSSION. The stimulus for the splenic contraction is unknown. However, Barcroft and Elliot (5), working on animals in heated chambers, observed contractions in normal spleens but none in denervated spleens. These results suggest a nervous mechanism. To compensate for the internal heat, there is a vasodilatation of the skin vessels in order to cause loss of heat by conduction (11). Thus, there is a shift of blood from the viscera to the periphery, which might account for the contraction of the spleen. It is also possible that the increased metabolism of the animal and the greater rate of circulation causes an "oxygen want" which might stimulate contraction.

There seems to be no alternative to the conclusion that the spleen is

directly responsible for the red cell concentration changes observed in these short duration fever treatments of dogs; and further, that this organ is, partly, or entirely, indirectly responsible for the concentration of plasma. The increase in serum protein with fever in normal animals and the absence of this increase in splenectomized animals, together with the results of the acute experiments cited above, tend to show that the addition to the circulation of concentrated red cells from the spleen has caused a displacement of fluid from the serum, possibly in order to maintain a constant blood volume.

These results, however, by no means rule out the possibility that the spleen is liberating concentrated plasma as well as red cells. Experiments in which splenic vein blood and splenic artery blood were drawn simultaneously before and after splenic contractions showed such insignificant changes in serum proteins that these experiments were discontinued. However, a recent paper by Goretsky and Ludany (11) shows that when all the vessels of the spleen except the splenic vein are tied off, and the splenic vein cannulated, contractions induced by faradic current applied directly to the spleen cause the liberation of blood containing plasma much higher in total nitrogen than the plasma of peripheral blood. The five experiments cited in their paper show an average increase in total nitrogen of 12.3 per cent over the normal. This result means that if the spleen liberated blood totaling one-third of the total blood volume (Barcroft), and if this blood consisted of 75 per cent cells and 25 per cent plasma, the addition of the serum liberated by the spleen would cause an increase in circulating serum of approximately 3-4 per cent. Therefore, even if this phenomenon occurs, it is not enough to explain the 10 per cent increases noted in our experiments. Thus, the mechanism most significant in the production of the blood changes there noted still seems to be the concentration of blood plasma resulting from the influx of stored erythrocytes expelled by the spleen.

CONCLUSIONS AND SUMMARY

1. Artificial fever induced in dogs by a G. E. inductotherm causes a significant contraction of the spleen.
2. This contraction is responsible for the observed polycythemia and the blood plasma concentration since neither of these effects occur in splenectomized animals.
3. Evidence is presented to show that absolute increase of red cells, produced experimentally, in acute dogs, by transfusion of blood corpuscles, results in increase of serum protein, due possibly to loss of water into the tissues.

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